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A MULTIVARIATE ANALYSIS OF THE PINUS CHIAPENSIS-MONTICOLA-STROBUS PHYLAD[1]

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As considered here, *Pinus chiapensis* (= *Pinus strobus* var. *chiapensis* Martinez) is the southern member of the North American pine phylad *Pinus chiapensis-monticola-strobus*. Phytogeographically, this complex forms a broad discontinuous triangle with apices of species distribution in British Columbia, Newfoundland, and Guatemala (Fig. 1).

Earlier, the taxonomic recognition of *Pinus strobus* var. *chiapensis* Martinez was questioned by several authors (Braun 1950: 483; Martin and Harrell 1957; Sharp 1946) who regarded it as a weakly-differentiated geographic form of *P. strobus* L. undeserving separate taxonomic rank, but others (Loock 1950: 117-119; Soto, Barrett, and Little 1962: 52-53; Standley and Steyermark 1958: 55-56) agreed with its varietal status. Gaussen (1960: 91, 198) proposed it as a distinct species but his new combination was not validly published because he omitted citation of the basionym (Art. 33, I.C.B.N., Lanjouw, *et al.* 1961).

These divergent opinions prompted the present biometric study to provide a statistical basis for a determination of the proper taxonomic disposition of this controversial taxon. In a companion paper (Andresen 1964), it is recommended that *P. strobus* var. *chiapensis* be elevated from varietal to

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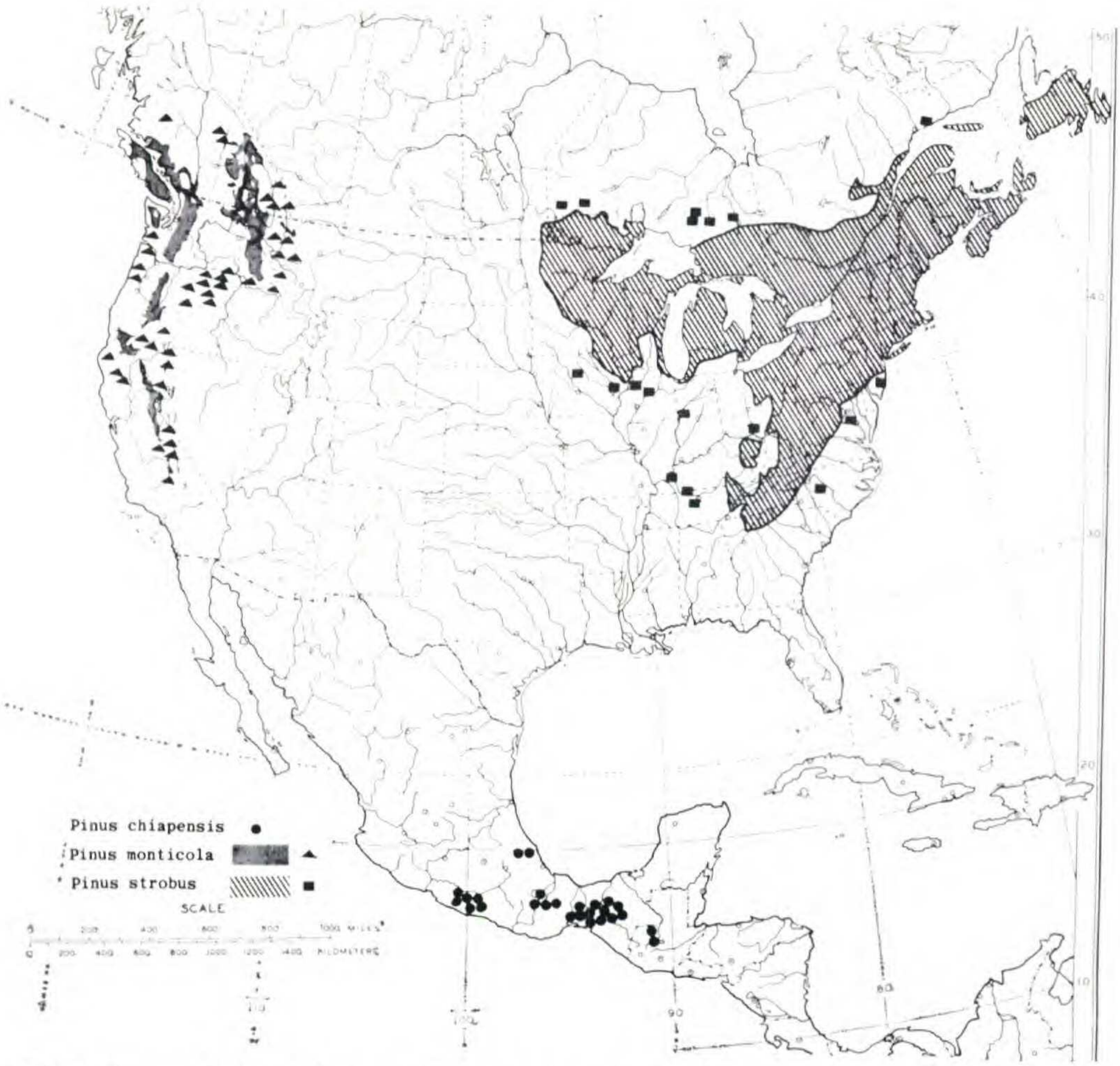

Fig. 1. — Distribution of the phylad *Pinus chiapensis-monticola-strobus* in Central and North America. Adapted from unpublished map supplied by U. S. Forest Service. Plotted on Goode's Base Map No. 202 by permission of the University of Chicago Press.

specific rank with the binomial *Pinus chiapensis* (Martinez) Andresen. The proposal was predicated on evidence obtained from the following study of morphologic and progeny data that were not available to previous authors. Following the analysis of this data, it became apparent that there was a much wider genetic and morphologic divergence than was previously suggested between *P. strobus* var. *chiapensis* and typical *P. strobus*.

Recent conceptual advances in the field of biosystematics (Heslop-Harrison 1963, Heywood and Love, 1963), that presented a clearer perspective of the problem at hand, led

Rogers (1963), and Sokal and Sneath (1963) to stress the value of reliable statistics that incorporate fundamental quantitative characters to determine taxa affinities or differentials. They coined the terms "taximetrics" and "numerical taxonomy" respectively, to focus attention on the statistical techniques designed to solve problems associated with experimental taxonomy. In this respect it is of primary importance to the taxonomist that contemporary statistical analyses and those yet to be derived can be programmed, especially with the aid of electronic computers, to assess the taxonomic value of any taxon trait or group of characters.

As an example, Davidson (1963) developed an "Itemized frequency distribution" and set of association indices based on the F statistic, to discriminate *Cirsium altissimum* from *C. discolor*. In a study of variability of the Pacific Coast and Rocky Mountain forms of *Pinus contorta*, Jeffers and Black (1963) used three forms of multivariate analyses which incorporated the "Q-technique," discriminant analysis, and component analysis, all of which rely on various manipulations of the correlation coefficient and its components. In this study, to show the degree of discrimination within the *Pinus chiapensis-P. monticola-P. strobus* complex, I employed the coefficient of divergence (*C.D.*) technique (Clark 1952) which is based on the sum of mean differentials of multivariate characters. To evaluate and assign relative importance values to the 12 leaf and cone characters used in the *C.D.* analysis, the F Statistic, intraclass correlation (expressed as a reliability index, R_I), and coefficient of variation were also used.

Data for the multivariate character analysis of cones, leaves, seed, and seedlings were obtained from herbarium and living specimens. Sample sizes, characters examined, statistical techniques, and pertinent examples to illustrate a particular method used are included as footnotes to data tables or in the discussion of the characters.

In the following text, P*inus chiapensis* is coded as *A; P. monticola* Dougl. as *B*; and *P. strobus* as *C*, except where the Latin binomials are more useful for clarity or emphasis.

COEFFICIENT OF DIVERGENCE VALUES

Before the importance of individual characters and their component roles in species delimitation within this phylad are evaluated, an explanation of the simultaneous treatment of fundamental characters is in order. A useful measure that incorporates this technique is the coefficient of divergence which is used in association with F statistics and reliability indices to summarize leaf and cone characters Q_1 to Q_{12} species pairs *AB*, *AC*, and *BC*. The method (Clark 1952, Klauber 1940) of arranging or ranking taxa in relation to the ascending magnitude of their *C.D.*'s is especially germane to studies utilizing objectively scored traits. Although Clark (1952) referred to herpetological data in his example, the extension of Klauber's (1940) one-dimensional coefficient by Clark is adaptable to any numerical or coded array of biologic parameters computed as a series of multiple character means.

In the present example (Table 1) four results are apparent: (1) About the same degree of information was provided by either 39 random samples or the total number of herbarium specimens examined (see Tables 1 and 2 to compare means of characters Q_1 to Q_{12} for the two sample sizes); (2) The *C.D.'s* derived from cone characters alone were greater in pairs *AB* and *AC*; (3) Leaf characters were more important than cone traits in computing the *C.D.* for *BC*; (4) With the incorporation of all 12 characters, *BC* is less than one-half as divergent as either *AB* or *AC*.

This magnitude of divergence, or lack of it, is of special significance — for according to the *C.D.* ranking, two long-recognized species, *P. monticola* and *P. strobus*, are much more closely aligned to one another than the species *P. strobus* is to its supposed variety *chiapensis*. This evidence, primarily the strength of the following F and R_I statistics

for the leaf and cone characteristics, plus other pertinent data, motivated the author to elevate *P. strobus* var. *chiapensis* to specific level (Andresen 1964).

LEAVES

Leaf length (Q_1) was greatest for *A*, but leaf width (Q_2) was least (Table 2). Thus, the resultant W/L value (Q_3) for *A* was smallest. Conversely, the shorter but wider leaves of *B* had a high W/L ratio that was twice as great as the value for *A*. An intermediate value of Q_3 for *C* is a reflection of the interjacent values of Q_1 and Q_2. The interpretation of this value, however, should be tempered by the lack of significance in the F statistic and the low reliability index of .497 for Q_2 in species pair *AC*; thus the component of greater relative importance for Q_3 in *C* is leaf length.

The role of leaf length in the ratio is emphasized because numerous authors (Loock 1950: 117; Martinez 1948: 133; Schwerdtfeger 1953; Sharp 1946; Soto, Vazquez, and Little 1962: 53) describe the leaves of *P. chiapensis* as "more delicate," "more slender," "finer," or "thinner" than *P. strobus*. These comparatives are misleading, however, when Q_1 and Q_2 are examined objectively. As shown earlier, there is a highly significant difference between *A* and *C* in regard to length but the width differential is non-significant. With these and other facts in mind, the following thoughts are offered to attempt an explanation of the reasoning of previous observers. In the field, the illusion of angustifoliation is probably created when observers familiar with leaf length in *P. strobus* subconsciously compare the much longer (and seemingly thinner) leaves of *P. chiapensis* and infer that the leaves of *A* are more delicate. But more important, because most descriptions are based on dry material, the gracile appearance of desiccated specimens is related to the herbarium artifact caused by severe dehydration and subsequent collapse of the leaf mesophyll. This condition was suggested when dried leaf specimens of *A* were noted to be deeply concave on all three surfaces (turgid leaves bear slightly convex surfaces), were curled

or twisted, and seemed lighter in weight than dried leaves of either *B* or *C*. To advance this postulation I (1) dried 125 leaves of *A*, *B*, *C*, and *B* X *C* grown under similar conditions near Placerville, California, and (2) calculated the proportionate weight loss of each set of samples by comparing fresh weight (weighed immediately after collection) to oven-dry weight. The differential percentages (Table 4) illustrate a much greater weight loss in *A* than the other three which were not significantly different from each other. Although tabulated at the 5% level of significance *A* was actually significant at the 2% level of confidence. The loss of 69% of the original weight in *A* is attributable mainly to the removal of fluids (mostly water) from the abundant intercellular space within the leaf mesophyll. A comparison of intercellular spaces within the leaves of the four taxa (Table 4) illustrates the significant differential between *A* and the other three.

Another feature which has previously been used to separate *A* from *C* is the supposed higher number of foliar resin canals in the former (Gaussen 1960: 91; Loock 1950: 117, Martinez 1940, 1947: 134; Soto, Vasquez and Little 1962: 53). This group of authors contends that the more common number of resin canals in *A* is 3 and more often 2 in *C*. The analysis (Table 4) however, provides data to the contrary. Based on 500 observations, *t* values at the 5% and 1% levels shows no significant differences between *A* and *C*. Mean values of 2.52 and 2.57 respectively, low standard deviations, and ranges of 2 to 3 resin canals for both species invalidate this character as a trait of differentiation. Although there is an overlap of range in number of resin canals between *B* and the former two, there is a significant difference between the means, so this feature could be used to separate *B* from *A* or *C*. Of interest is the broad range of numbers of resin canals in the hybrids of *B* and *C* and the large standard deviation, but this is an established pattern with numerous hybrid pines (Keng and Little 1961).

Even though the original description (Martinez 1940) of *A* was predicated on the criteria of thinner leaves and a greater number of foliar resin canals than *C*, the data indicate that both characters are of little or no value in separating the two taxa.

On the other hand, a character apparently overlooked in the past that has high diagnostic utility is the degree of leaf serration, Q_4 (Table 2). In fact, the F value for this character for species pairs *AB* or *AC* was the most significant of any leaf parameter. Twice as many serrations per 5 mm interval were found on *A* when compared to *C*. The high reliability index of .995 for Q_4 of pair *AC* also demonstrates the dependability and high level of confidence to be expected when using this trait for species separation. Also, a low coefficient of variability of 14.3 for *A* in contrast to 25.4 for *C* and 57.5 for *B* is added measure of reliability for this character in *A*. In a progeny study of juvenile forms of *P. strobus*, Mergen (1963) found an increase in serration number in relation to progression from southern to northern sources of origin. We found just the opposite trend in mature field specimens, and observed a random pattern in juvenile specimens.

The weakest leaf character of the five examined (Table 2) was the number of stomatal rows borne on the ventral (adaxial) surface, Q_5. Although there was a significant difference between *A* and *C* for Q_5, the reliability index was lowest in comparison to the other significant features of leaf characters. Stomata on the dorsal (abaxial) surface, although not included in the character analysis, were absent on all 440 leaves examined of *A* and all 3408 leaves examined of *C*. Dorsal stomata were present, however, on *B* leaves with a mean of 0.28 and a range of 0 to 4 based on a 1279 leaf-count. When present, this is a reliable character to separate *B* from *C* (Harlow 1947, Sargent 1897: 23, Shaw 1914: 34). In two studies of pine leaves however, (Doi and Morikawa 1929, Sutherland 1934) reported an absence of dorsal stomata in *B*.

Fig. 2. — Comparison of cone characteristics of *Pinus chiapensis* (*A*), *P. monticola* (*B*), and *P. strobus* (*C*).

PLATE 1318

CONES

Since the value of the mean for ovuliferous scale number (Q_6) was highly dissimilar between the three species and *C.V.*'s were relatively small, large F statistics and strong reliability indices were derived. The highest number of scales were found on *B* (Fig. 2, Table 2) and even though there were about 25% more scales per cone in *A* than in *C*, cone lengths were not significantly different. Precise cone-phyllotaxis of the three species was very difficult to determine in mature open cones, so this trait was ignored.

Shaw (1914: 12) observed that this pattern of cone scale arrangement presented an indefinite phyllotaxy for the sub-genera Diploxylon and Haploxylon and the only importance of phyllotactic differential is that it separates the two sub-genera on the basis of a higher order-fraction in the former group. Nevertheless he commented that in cones of equal size, *B* had an obviously higher phyllotaxy than *C*. The phyllotaxy of *A* was also higher than *C* but lower than *B*.

Apophysis width (Q_7) in both *A* and *C* was similar, but length (Q_8) was more divergent, (Table 2, Fig. 2). Even though Q_7 and Q_8 were dimensionally greater in *B* than in the other two taxa, the resultant ratio (Q_9) of the three species was largest for *A* because of its higher proportionate width. A high F statistic and reliability index of .959 of Q_9 for *AC* indicate the potential diagnostic value of this trait. In pair *BC*, the ratio differential was non-significant with a low R_1, and so was of little value in separating the cones of *B* and *C*.

Length of peduncle (Q_{10}) was greatest in *A*, but shorter and about the same for *B* or *C*. Although F and R_1 values of Q_{10} were high for pairs *AB* and *AC*, the low values in association with *BC* indicate the similarity of this trait in *B* and *C*. Character Q_{10} is, at times, difficult to measure for the peduncle is brittle and tends to fracture easily. Caution must be exercised to carefully collect and store the cones to avoid negation of this character.

Another reliable cone trait to isolate *A* from *B* or *C* is the degree of scale margin undulation (Q_{11}). In *A*, the apophysis margin with inflexed umbo-tip forms several involutions (Fig. 2) rarely found in *B* or *C*. As with peduncle length, scale margin bore differentially high statistics for pairs *AB* and *AC*, but was useless to segregate *B* from *C* in which the umbos were simply concave. Martinez (1940) and Soto, Barrett, and Little (1962: 53) described the umbo as having ". . . undulating edges turned inward." but in their work this conspicuous feature was not com-

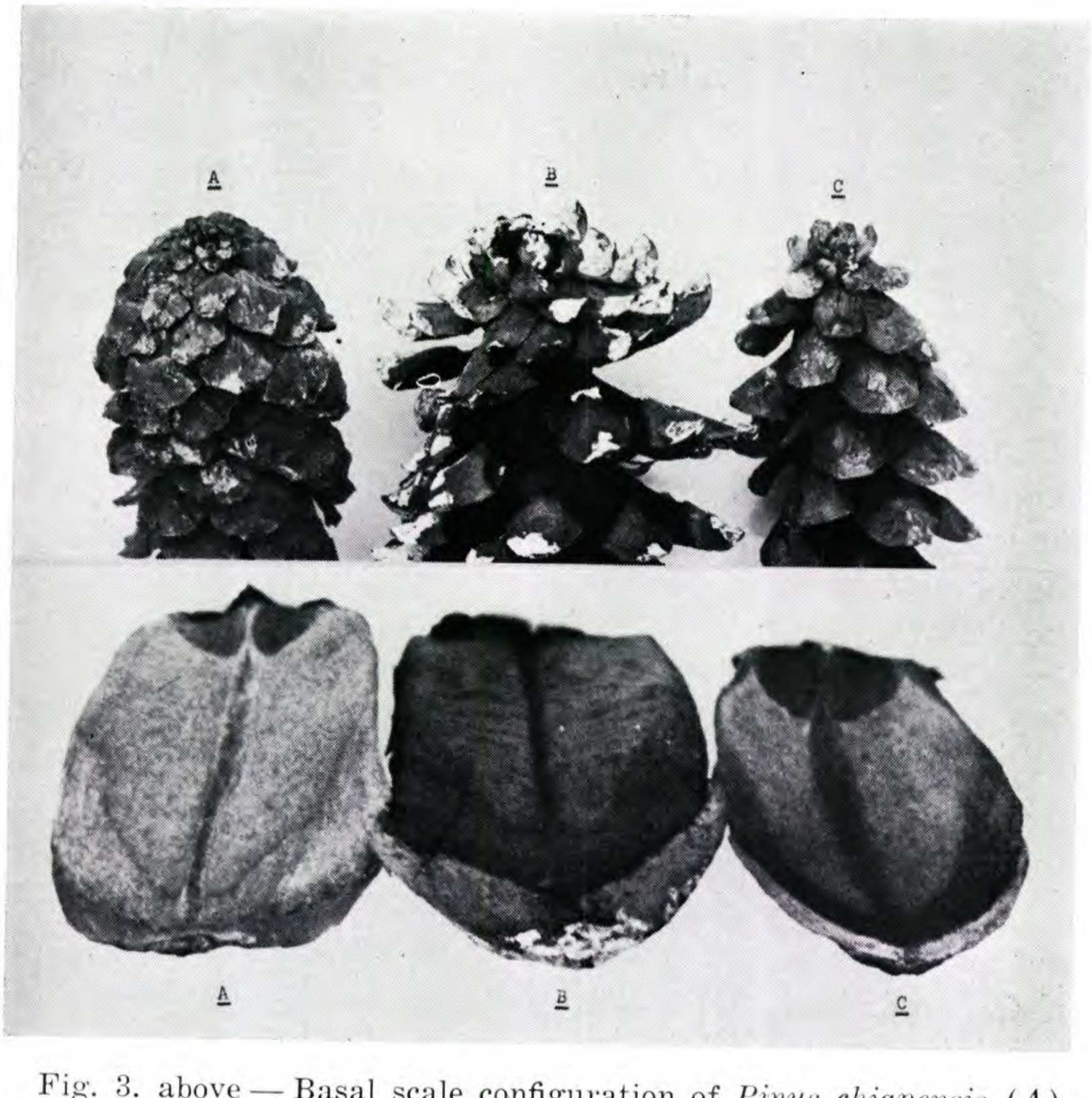

Fig. 3. above — Basal scale configuration of *Pinus chiapensis* (*A*), *P. monticola* (*B*), and *P. strobus* (*C*).

Fig. 4. below — Ovuliferous scales (adaxial surface) of *Pinus chiapensis* (*A*), *P. monticola* (*B*), and *P. strobus* (*C*).

PLATE 1319

pared to other species. Also, the scale apex of *A* is truncate versus the rounded apices of *B* and *C* (Fig. 4).

The most outstanding difference in cone morphology and the character that was related to the highest F and R_1 values of all leaf and cone characters for pairs *AB* and *AC* was the relative number of basal reflexed scales (Q_{12}). This curving of scales contiguous to the peduncle of taxa *A* and *B* is obvious in numerous illustrations (Gaussen 1960: 199; Harlow and Harrar 1958: 57; Sargent 1897: plates 539, 541; Sudworth 1908: Fig. 2). In Fig. 3,

a comparison of the number and presence of these scales in *B* and *C* is made with the lack of reflexing in *A*. In our samples of *A*, none of the cones bore basal scales that were fully reflexed, and only a few were weakly curled. Since *F* and R_1 values were high for all three pairs, an examination of Q_{12} alone would suffice to determine individual members of the phylad.

In addition to the foregoing values obtained from leaf and cone measurements, the data following the discussion of sample size also add confirming evidence that *P. chiapensis* is strongly divergent from *P. strobus*.

SIZE OF SAMPLE

In biometric analyses, achievement of the highest degree of precision commensurate with judicious use of time is a goal of primary importance. In Table 1, it is demonstrated that approximately the same coefficient of divergence can be calculated by measuring relatively large numbers of specimens or by a less time-consuming random sample of 39. The question then is how many samples are required to provide a desired level of accuracy in separating these taxa? Numerous models and examples are offered by Cochran and Cox (1950), Dixon and Masey (1957), and Snedecor (1956), but I prefer the technique derived by Wright and Freeland (1960). Their method, in which it is advantageous to use small sample numbers, employs standard deviations and means of the components of any species pair. When substituted in the formula in Table 3, these values multiplied by an appropriate "t" provide a predictable sample size for any required level of accuracy. Since the minimum number of complete samples within *A*, *B*, or *C* was 39 and a recommended minimum sample (Freese 1959) should approximate 30, we used a set of 39 observations for the 12 characters to obtain the s, x, *F*, and R_1 data in Tables 2 and 3. Also, from past experience with the group *Strobi*, it seems that 30-40 random samples are required for an initial character survey. Estimates of sampling (Table 3) for further studies of characters Q_1 to Q_{12} for taxa pairs

AB, *AC* and *BC* indicate that a relatively small number of specimens is required to exhibit statistical significance for most traits. A maximum of ten samples of each species within a pair would be required to detect a difference at the 5% level of confidence in 28 of the 36 possible character-taxa pair combinations and 20 samples to detect differences at the 1% level for 28 out of 36. These are reasonable sample sizes with which to work, and the number of characters, if definitive, could be expanded to give a more precise evaluation of a total difference. Note the direct relationship (Table 3) between the reliability index and the number of samples required. Low or negative R_I values correspond to higher sample numbers. Large sample sizes, e.g. 1352 and 227 for *BC*-Q_{10} and Q_{11} would require a prohibitive amount of observation time, so these characters are best not measured.. The time saved could be better spent on other observations such as those which follow.

SEED AND SEEDLING CHARACTERISTICS

Differences in seed weight of the three species used in the progeny tests are given in Table 5. To determine the accurate weight of sound seed each lot was first floated in 95% ethyl alcohol to separate the denser, sound seed from the buoyant, partially filled seed. After winnowing, the seeds were floated and it was discovered that about 10% of the seed were blank. Based on this unit of malformed seed and experiences with the other group *Strobi* seed, we converted the reported weights (Anon 1948: 269) of *P. monticola* and *P. strobus* to arrive at 53,460 per kg which compared very closely to our data for the two species. The large number of 60,000 seed per kg for *A* is related to the smaller size of the individual seed. The smaller seed may be linked to the ability of propagules with reduced endosperm to survive in the favorable growing and germinating conditions within the natural range of *A*.

The seed of all three species, in either the stratified or unstratified condition, began to germinate within 10 to 16 days after sowing (Table 5). In contrast to *B* and *C*, the

germination period for *A* had ceased 37 days after initiation of the test — also, the germination values for either treatment were identical. On the other hand, unstratified seed of *B* and *C* had considerably lower values than those stratified and some seed of both treatments had not germinated at the end of the test. The marked contrast in germination values between *A* and *B* or *C* is related to a major physiological difference within the phylad germination regime.

Under uniform growing conditions in a greenhouse the seed sources of *A* produced seedlings with longer hypocotyl and cotyledon length. *A* was significantly larger than *B* and *C*, but *B* was smallest. Larger numbers of samples are required to determine the variability within *B* and *C*, but the 30 sources of *A* seemed adequate to assess some infraspecific variation. Cotyledon numbers were not significantly different from one another although *C* had a slightly higher range and mean. Englemann (1880) reported 6-9 for *B* and 7-11 for *C*. Of all the seedling characteristics examined in this study, cotyledon number was most uniform and of least diagnostic value.

Shaw (1914: 1) suggested that cotyledon morphology and number are unreliable for species identification unless there are distinct numerical differences between species. In a recent study of the *Pinus flexilis - P. strobiformis* complex (Andresen and Steinhoff 1965), a significant difference was found in numbers of cotyledons with a mean of 8.8 for *P. flexilis* and a mean of 11.5 for *P. strobiformis*.

In addition to the above seedling characteristics, there was a difference in growth of secondary leaves and bud setting. As might be expected, because of its southern origin (Mirov 1962), *A* produced secondary leaves much earlier than either *B* or *C*. No secondary leaves were formed on either *B* or *C* during the first 200 days of observation. Also, *A* did not produce any dormant buds, for as soon as a bud was formed it produced continuous growth of leaves. Definite dormant buds did form on *B* and *C*. In the course of my

field work in December 1962, I observed numerous *P. chiapensis* south of Sola de Vega, Oaxaca, Mexico, which had produced several increments of cones the past growing season and with a few trees undergoing anthesis. Mr. Boone Hallberg (personal correspondence) has noted this phenomenon for several years and reports that an average stand in Oaxaca has the following periodicity of cone maturation:

Percent of Trees	Date of Maturation
20	20 July + second crop about 20 November
10	1-20 August
60	25 August - 15 September
10	15 September - 10 October

Mirov (1962) also observed that *P. oocarpa* in Nicaragua and other tropical pines are characterized by accelerated and uninterrupted growth rhythms. Only one year is required for seed set after pollination.

CONCLUSIONS

Coefficient of divergence values indicate a much closer morphological affinity between *Pinus strobus* and *P. monticola* than between *P. strobus* and the former variety *chiapensis*. The wide divergence between *P. chiapensis* and *P. strobus* provides one form of biometric evidence to argue against the opinions of earlier writers advocating that the two taxa should be conspecific. With the available *C.D.* values and the sustantiating data that follow, little doubt remains that *P. chiapensis* is a distinct species. An analysis of the more statistically significant characters that discriminate *P. chiapensis* from *P. strobus* reveals that *P. chiapensis* differs by having: (1) 25% more leaf serrations per unit length, (2) leaves that are 31% longer, (3) cone-scale apophyses that are truncate and 20% shorter, (4) extremely thin and wavy apophysis margins, (5) no reflexed scales contiguous to the peduncle, (6) 13% more seed per kg, (7) seed that germinates rapidly and uniformly without after-

ripening, and (8) continuous growth in the primary seedling stage without formation of dormant buds.

Ecologically, *P. monticola* and *P. strobus* grow within similar sub-boreal to cool temperate habitats. Although there undoubtedly are clinal or ecotypic variants (Hanover 1962; Mergen 1963; Wright, Lemmien and Bright 1963) within the geographic distribution of the two species, three omnipotent growth factors are present: (1) both species are influenced by annual photoperiodic cycles of short and long days that occur between 34° and 52° N. Lat., (2) precipitation ranges from 500 to 2000 mm (with up to 3450 in Coastal Washington) with pronounced dry periods during the summer months, and (3) temperatures are low enough to induce marked winter dormancy of three to six months. On the other hand, *P. chiapensis* is subjected to sub-equatorial insolation from 15° to 20° N. Lat. and is under the influence of a humid, warm-temperate climate associated with abundant precipitation concentrated in the summer months. In addition, this effective precipitation is further enhanced by frequent mountain fogs. Leopold (1950) described the *P. chiapensis* phyto-association as a tropical cloud forest while Beard (1944) termed it a temperate rain forest. Even though most *P. chiapensis* forests are limited to subtropical or warm-temperate climates there are exceptional isolated stands that occasionally are exposed to freezing temperatures.

In addition to the foregoing morphological and ecological divergences there also are ancient phytogeographical divarications that are extremely important when considering the origin of *Pinus chiapensis*. I believe that the original dispersal of the phylogenitors of *P. chiapensis*, *P. monticola*, and *P. strobus* was accomplished at least by early Eocene and that later but similar forms, morphologically and physiologically allied to the three contemporary taxa, were isolated from one another by late Pliocene. My reasoning follows:

Substantial evidence (Gausen 1960) of an ancient Hap-

loxylon pine flora richer and more diverse than the subgenus now extant has been revealed in the fossil record of the late Mesozoic and Arcto-Tertiary forests. With the advent of accelerated climatic change in the pre-Pliocene (Good 1953: 263), xeric climatic bands evolved (Schwartzbach 1963) that were unfavorable to the mesophytic plants of the southern portion of the Arcto-Tertiary biome. These latitudinally oriented zones contributed to the formidable hiatus which first isolated the early Quaternary progenitors of such contemporay pine relicts as *Pinus ayacahuite*, *P. chiapensis*, *P. griffithii*, *P. parviflora*, and *P. peuce*. In the early Pliocene of North America pronounced orogenesis contributed to a secondary discontinuity that further divided the forests above 25° North Latitude into eastern and western disjuncts. It was then, in the forested and mountainous areas of the Appalachians, the Pacific North West, and the southern Mexican-Guatemalan highlands, that the immediate ancestors of *P. chiapensis*, *P. monticola*, and *P. strobus* persisted in competition with their associates.

Thus, prior to the continental and montane glaciation of the Pleistocene, isolation of the phylad segregates was complete and to this day final. The waves of subsequent Pleistocene ice with their periglacial climatic regimes undoubtedly caused areal concentration of both *P. monticola* and *P. strobus*. However, the cooler temperatures and neopluvial conditions that were contemporary with glaciation did not, as Deevey (1949), Dressler (1954), and Sharp (1953) suggest, create an ameliorated environment across the semi-arid Texan hiatus that would have permitted the mesophytic phyto-associations of the southeastern United States to reach and penetrate the montane regions of eastern and southern Mexico.

Martin and Harrell (1957) observed that further detailed ecologic and taxonomic revisions should be made of the vicariads of these two biotas. For only with new evidence can the theory of mid-Cenozoic disjunction be expanded.

Finally, the divergence patterns and biometric differentials within the Holocene *Pinus chiapensis-monticola-strobus* phylad provide one such example of floristic confirmation of pre-Pleistocene isolation of the montane forests of southeastern Mexico, as suggested by McVaugh (1952); Martin and Harrell 1957; and Steyermark 1950.

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Table 1. Computation and Comparison of Coefficients of Divergence within the *Pinus chiapensis* — *P. monticola* — *P. strobus* Complex. Based on Means[1] of Characters Q_1 to Q_{12} for Taxa *A* to *C*.

Taxa	Parameter Means											
	Q_1	Q_2	Q_3	Q_4	Q_5	Q_6	Q_7	Q_8	Q_9	Q_{10}	Q_{11}	Q_{12}
A	104.6	0.65	0.0062	12.9	4.3	87.7	17.7	9.9	1.79	22.4	2.5	0.04
B	68.1	0.92	0.0135	3.1	4.0	110.8	20.0	14.2	1.41	16.1	1.0	13.10
C	72.7	0.67	0.0092	6.1	3.4	67.6	16.4	12.4	1.32	16.4	1.1	7.00

Coefficients of Divergence, C. D., derived from the above data and means of 39 randomly selected samples from same populations values:

Taxa with all samples	with 39 samples			species pair			
	Leaf	cone	aggre-gate	leaf	cone	aggre-gate	
BC	.187	.155	.169	.189	.145	.165	*P. monticola* vs. *P. strobus*
AC	.206	.414	.343	.207	.418	.346	*P. chiapensis* vs. *P. strobus*
AB	.343	.424	.392	.348	.423	.393	*P. chiapensis* vs. *P. monticola*

[1] In the above summary of means let:
A denote 60 foliage samples and 40 cone samples of *Pinus chiapensis* collected from individual trees found in southern Mexico, and northern Guatemala.
B denote 120 foliage samples and 45 cone samples of *Pinus monticola* collected from individual trees found in southwestern Canada and the northwestern United States.
C denote 300 foliage samples and 47 cone samples of *Pinus strobus* collected from individual trees found in the Lake States, and the northeastern and southeastern United States,
and let following Q_1 to Q_{12} represent:

Q_1 total leaf length in mm
Q_2 leaf width in mm
Q_3 ratio of leaf width to leaf length Q_2/Q_1
Q_4 number of serrations per 5 mm interval of leaf edge at leaf center

Q_5 number of stomatal lines on vertral leaf surface
Q_6 total number of ovuliferous scales of mature cone
Q_7 width of scale apophysis in mm
Q_8 Length of scale apophysis in mm
Q_9 ratio of apophysis width to length Q_7/Q_8
Q_{10} length of cone peduncle in mm
Q_{11} involutions of edges of central scales, range from 0 to 4
Q_{12} number of reflexed basal-scales contiguous to peduncle; range from 0 to 18

To calculate any C. D., the following general formula was used:

$$C.D._{AC} = \sqrt{\frac{(a_1 - b_1)^2 + (a_2 - b_2)^2 + \ldots + (a_{12} - b_{12})^2}{k}}$$

where: $a_1 = \frac{A_1}{A_1 + B_1}$; $b = \frac{B_1}{A_1 + B_1}$ for Q_1 etc.

k = no. of characters, or 12 in this study

Table 2. Means and standard deviations of characters $Q_1 \ldots Q_{12}$ for *Pinus chiapensis* (*A*), *P. monticola* (*B*), and *P. strobus* (*C*), derived from 39 random samples. F statistics for species pairs *AB*, *AC*, and *BC* incorporate 1 and 38 d.F.

Character	*Pinus chiapensis*		*Pinus monticola*		*Pinus strobus*		F Statistics[1]		
	$\overline{X}$	s^2	$\overline{X}$	s	$\overline{X}$	s	*AB*	*AC*	*BC*
Q_1	106.51	14.44	69.21	16.42	77.97	12.29	113.51	88.30	7.13
Q_2	0.66	0.06	0.95	0.12	0.68	0.08	203.47	2.97	142.09
Q_3	0.0062	0.0008	0.0148	0.0042	0.0089	0.0013	154.83	121.90	69.46
Q_4	12.92	1.84	3.15	1.84	5.69	1.45	548.54	370.60	45.61
Q_5	4.33	0.83	4.09	0.89	3.46	0.77	1.46	23.20	11.41
Q_6	87.72	9.08	114.95	29.68	68.36	12.90	30.03	58.76	80.85
Q_7	17.74	1.82	20.10	3.14	16.49	1.50	16.53	11.08	42.17
Q_8	9.95	1.15	14.33	2.14	12.54	1.74	126.87	60.02	16.44
Q_9	1.80	0.22	1.41	0 23	1.33	0.17	55.03	106.02	2.75
Q_{10}	25.10	5.84	15.77	4.66	16.10	3.10	60.82	72.26	0.14
Q_{11}	2.43	0.72	1.10	0.31	1.05	0.22	113.66	132.23	0.71
Q_{12}	0.03	—	12.54	2.39	7.41	1.63	1064.13	794.19	122.13

[1]Values under 2.98 are non-significant. Value of 7.13 is significant at 5% confidence level. Values greater than 11.07 are significant at 1% confidence level.

[2]The coefficient of variation (C. V.) discussed in the text is calculated from the formula C. V. $= s^2\overline{X}$ (100). For example in A — Q C. V. $= (14.44)^2/106.51$ (100) $= 13.6$

Table 3. Character reliability indices (R_1)[1] and number of samples[2] of each taxon required to detect significant differences at the 5% and 1% level within species pairs *AB*, *AC*, and *BC*.

Character	Reliability index			Samples needed to detect differences					
	AB	*AC*	*BC*	*AB*		*AC*		*BC*	
				5%	1%	5%	1%	5%	1%
Q_1	.983	.978	.754	3	5	4[3]	6	22	39
Q_2	.990	.497	.986	2	3	50	82	3	4
Q_3	.987	.984	.972	2	4	3	4	5	6
Q_4	.996	.995	.957	2	3	2	3	7	8
Q_5	.189	.917	.839	99	157	8	14	15	26
Q_6	.936	.965	.976	7	11	6	7	5	6
Q_7	.883	.835	.954	10	17	16	27	7	8
Q_8	.984	.967	.885	3	4	5	7	10	17
Q_9	.964	.959	.288	6	7	4	5	68	108
Q_{10}	.968	.973	−.758	6	7	5	6	1352	2160
Q_{11}	.983	.985	−.169	4	5	3	4	227	354
Q_{12}	.998	.997	.984	1	1	1	1	3	4

[1]Although R_1, as the intraclass correlation, is more often used to compare characters between genetically related individuals, it also serves here as a statistic to evaluate character weight. It is computed from the model $R_1 = \sigma^2_B / (\sigma^2_B + \sigma^2_W)$ where σ^2_B is the between taxa component of the total variance and σ^2_W is the within component of the total variance for any one character. In AB—Q_1, .983 means that 98.3% of the proportionate variance is related to differences between taxa rather than within taxa. High R_1 values indicate "strong" characters of differentiation.

[2]Sample sizes were determined by solving for n in the formula $n = 2\,Vt^2/(\bar{X}_1 - \bar{X}_2)$ which was derived from the model of "student's" t. In our sense, n = degrees of freedom or sample size, V = pooled variance of a species pair, t=estimated value from "student's" t-distribution, $\bar{X}_1 + \bar{X}_2$ = means of character X in species 1 and 2 respectively.

[3]To calculate the number 4 proceed as follows: The variance (σ^2) of Q_1 for *A* is 207.36 and for *C* is 151.29 (pooled $V_1 + V_2$ should be divided by 2), the mean leaf length for *A* is 106.5 and 78.0 for *C*; these values substituted in the n formula give: $n = 2/2\ (358.65)t^2/(28.5)^2 = .442t^2$. Since .978 is a high R_i and indicative of a low n, substitute n = 2 to find an empirical t; ($t = 2n - 2$):

with 2 df
$2 = .442t^2$
t for 4—2 df
at 5% = 4.30
$2 = .442(18.50)$
$2 = 8.18$
but $2 \ddagger 8.18$

so try 6 df
$6 = .442t^2$
t for 12—2 df
at 5% = 2.23t
$6 = .442(4.97)$
$6 = 2.20$
but $6 \ddagger 2.20$

so try 3 df
$3 = .442t^2$
for 6—2 df at 5% = 2.78
$3 = .442(7.73)$
$3 = 3.42$ rounded = 4
so the conservative n = 4

with 4 df the "t" side was only 2.63, so the appropriate n is reached the first time the "t" side of the equation exceeds the "n" side.

Table 4. Internal leaf characters: number of resin canals and succulence represented by percents of moisture and intercellular space of *P. chiapensis*, *P. monticola*, *P. strobus*, and hybrid *P. monticola* X *strobus* (*D*).

Taxa	Resin Canals Mean No.	Std. dev.	Range	Taxa	Succulence % Moisture	% Intercellular sp.
D	1.89[1]	.581	0-4	A	67.75**	40**
B	2.25	.266	2-3	*B*	58.89	10
A	2.52	.207	2-3	*C*	58.76	10
C	2.57	.176	2-3	*D*	58.26	10

[1]Taxa *D* and *B* or *A* and *C* not significantly different from each other in number of resin canals but former pair is significantly different from latter at 1% level using the value of t.

**Taxa *A* values significant at 1% level, *B* to *D* non-significant.

Table 5. Seed and Seedling Characteristics of *Pinus chiapensis*, *P. monticola*, and *P. strobus*.

Taxa	Seed per kg	Seed Germination rate in days		Germination values[3]	
$\overline{X}$	Range	Unstrat.[1]	Strat.[2]	Unstrat.	Strat.
A 61,100	46,900 to 104,200	10 to 37	10 to 37	10.35	10.35
B 52,300	39,400 to 67,800	10 to 186+	13 to 186+	0.04	0.48
C 53,800	47,000 to 66,700	16 to 186+	13 to 186+	0.48	1.28

[1]Unstratified seed stored dry at 4°C. for 60 days.

[2] Seed stratified in moist peatmoss at 4°C. for 60 days.

[3]Germination value = greatest cumulative number of seed that germinated in fewest days multiplied by total percent germinated, divided by total number of days in test period (McLemore and Czabator 1961).

	Seedlings					
	Hypocotyl Length mm		Length mm		Cotyledon Number	
	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range
A	39	20 to 70	28	20 to 40	8	6 to 10
B	20	15 to 35	18	14 to 25	8	7 to 10
C	31	18 to 44	25	15 to 30	10	8 to 12